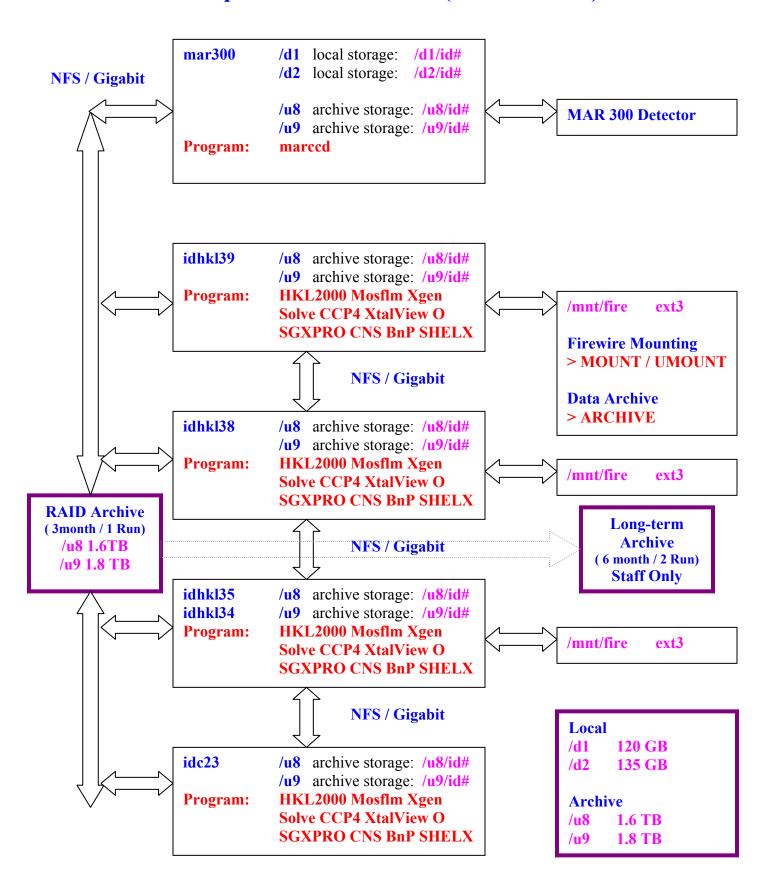


SER-CAT Tutorial

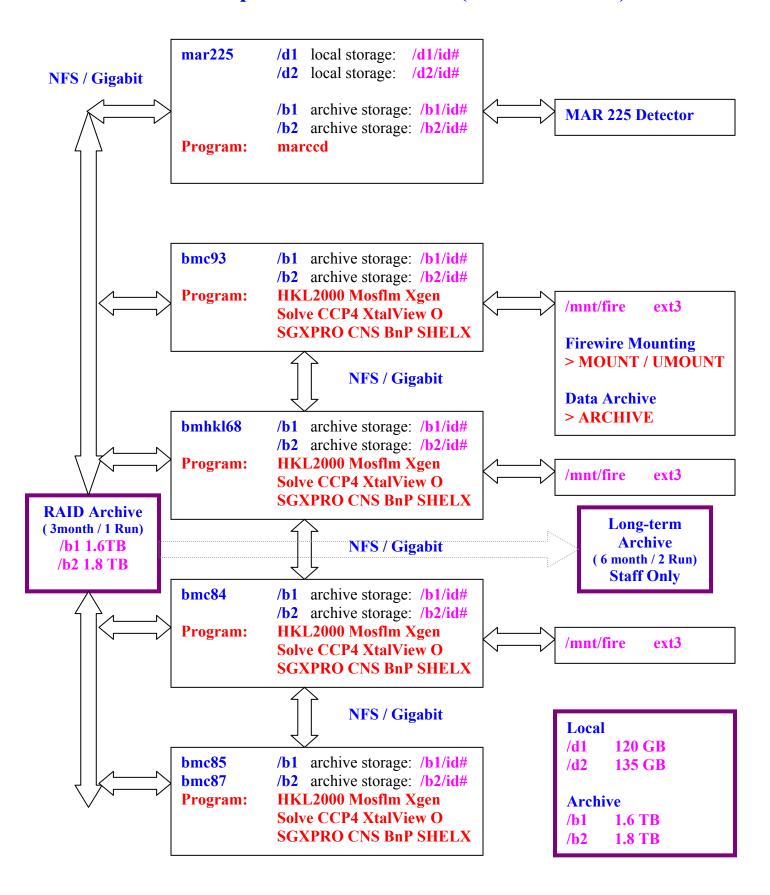
- **Computer Disk Structure (ID Beamline)**
- **Computer Disk Structure (BM Beamline)**
- **Startup Procedure**
- ***** Firewire Drive Mounting Procedure
- **Data Archive Procedure**
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Latest Update: January 30, 2006 V5.0

Computer Disk Structure (ID Beamline)



Computer Disk Structure (BM Beamline)



Startup Procedure

1. Log into beamline control computer with assigned username and password.

ID beamline: idc23 (IP: 164.54.208.23)
BM beamline: bmc93 (IP: 164.54.208.93)

- 2. Start MARCCD program.
 - a. Start shell window from idc23 for ID or bmc93 for BM

b. **\$ marccd** (This script will automatically marccd program remotely)

- 3. Start **SERGUI** program.
 - a. Start shell window from idc23 for ID or bmc93 for BM

b. \$ sergui (most common functions)

- i. >Hutch (wavelength, slit size, transmission, optimization, distance, etc)
- ii. >Sample (click and alignment, autoalignment, fine adjustment)
- iii. >Collect (collect single, collect strategy, collect run)
- iv. >MAD (periodic table, energy fluorescence scan, f" and f' analysis)
- c. Create data archive directory from > Collect > Archive Directory > Browse

Example template: /u9/staff/08 08 05 SERCAT staff

- **4.** Start **MOTOR** program.
 - a. Start shell window from idc23
 - b. \$ motor
- 5. Mount firewire drive (file system: **EXT3**) as /mnt/fire into one of following computers by following Firewire Drive Mounting Procedure:

ID beamline: idhkl34, idhkl35, idhkl38, idhkl39 BM beamline: bmhkl68, bmc84, bmc85, bmc87

6. Start automatic data archive script from the machine with firewire drive mounted by following **Data Archive Procedure**.

Example:

From directory: /u9/staff/08 08 05 SERCAT staff

To Directory: /mnt/fire

- 7. Start HKL2000 program.
 - a. Start shell window from idc23, idhkl34, idhkl35, idhkl38, idhkl39 for ID beamline.
 - b. Start shell window from bmc93, bmhkl68, bmc84, bmc85, bmc87 for BM beamline.
 - c. \$ HKL2000

Firewire Drive Mounting Procedure

- 1. Several beamline data processing computers (idhkl38, idhkl39, idhkl35, bmhkl68, bmc84) have the capability for firewire drive mounting.
- 2. From beamline staff and users' experience, we found out that Firewire drive with **vfat** file system is very unreliable and easy to corrupt.

To protect user's precious data, we will only support user's Firewire drives with file system ext3.

3. idhkl34, idhkl35, idhkl38, and idhkl39, bmhkl68, bmc84, bmc85, bmc87 are configured to mount firewire drive ONLY with file system ext3.

Mounting point: / mnt / fire

Mounting steps:

a. To mount: \$ MOUNT (Notice: Upper case cmd and follow instruction)

b. To umount: \$ UMOUNT

- 4. Procedure to reformat user's firewire drive to ext3.
 - 1) Login as root.
 - 2) Connect firewire drive to power supply and wait for the front light turn to green.

clean up the scsi bus

3) Connect firewire cable to the computer.

4) % rescan-scsi-bus -r

```
5) % /sbin/fdisk /dev/sda # reformat the drive
d # delete old partition
n # ask for a new partition
p # specify a primary partition
1 # pick cylinder 1 to begin with
w # write out the new partition table
```

6) % /sbin/mke2fs -j -m0 -LNAME /dev/sda1 # make a filesystem

Data Archive Procedure

Procedure

From data processing computer idhkl35, idhkl38, idhkl39, bmhkl68, bmc84

MOUNT # start automatic **MOUNT** script to mount firewire drive

% ARCHIVE # start automatic ARCHIVE scrip to backup data

From directory: /u9/staff/08_08_05_SERCAT_staff
To directory: /mnt/fire # user input: from archive directory # user input: to mounting point

Notice: ARCHIVE script will keep running every 5 minutes, and will automatically backup user's data from /u1 or /u2 to firewire drive incrementally.

Ctrl^C # stop ARCHIVE script after finishing data backup

% UMOUNT # start automatic **UMOUNT** script to unmount drive

Policy

- 1. Before user leaves the site, it is user's responsibility to backup experimental data and to inform SERCAT staff of the whereabouts of archived data in /u8 and /u9 RAID disk.
- 2. User should follow Firewire Drive Mounting Procedure to mount / unmount firewire drive.
- 3. Archived data will be kept in /u8 or /u9 RAID disk for about 3 months (1 run).
- **4.** CAT staff will then archive data from /u8 or /u9 RAID disk to a long-term storage system (staff access only). The archived data will be kept in long-term storage for about 6 months (2 runs).
- 5. Due to tightened APS firewall policy, user is not able to access data processing computers remotely. To retrieve archived data in the future, user should provide staff user's institution ftp server IP address, user name, password and large disk space.
- **6.** Occasionally, user may borrow a loan Firewire drive (pre-formatted and 160 GB) from SERCAT. Please leave user name, institution name, and contact information on the assigned log book; and return the drive to staff as soon as possible.

Energy Change and Beam Alignment Procedure

Any energy change between 6.2 Kev (2 A) and 13.5 Kev (0.92 A)

- 1) Change energy from sergui window.
- 2) Enable the experimental hutch and open safety shutter.
- 3) From sergui > hutch tab, run following optimizations.

Optimize Tune

Program will automatically run scans until the Gaussian peak is found. Program will automatically move to optimized peak.

No user interaction is necessary.

Optimize Horizontal Slit

Program will automatically run scans until the Gaussian peak is found. Program will automatically move to optimized peak.

No user interaction is necessary.

Optimize Vertical Slit

Program will automatically run scans until the Gaussian peak is found. Program will automatically move to optimized peak.

No user interaction is necessary.

If user wants to do large energy change, for example, from 12 kev to 6 kev, user may continue

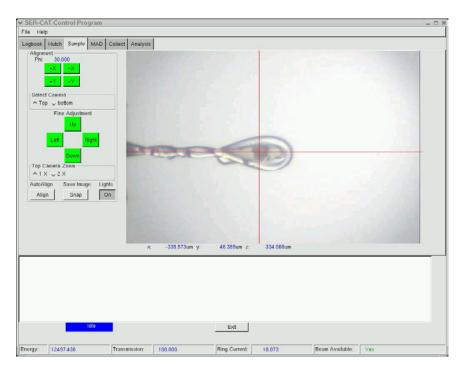
- 4) Put on the phosphor ball provided by CAT staff and align the ball to the center.
- 5) Tweak the goniostat position to bring the spindle center to beam center
 - i. Reduce slit size to **20x20** and reduce beam transmission to have better view of the beam center profile.
 - ii. From motor> window, type command

```
motor> mrel g_hor 10 (+/- 10 to +/- 100 movement)
motor> mrel g vert 10 (+/- 10 to +/- 100 movement)
```

iii. After align the beam center to the upper horizontal line of cross box, close the timing shutter, increase slit size to normal 100, and set filter to desired number

Crystal Alignment Procedure

SERGUI has been modified to provide automatic loop centering. The system still supports the point and click alignment feature that has been in place for some time.



Auto-Alignment

The AutoAlign button has been activated. Auto loop centering can be performed with either the top (both magnifications) or bottom camera. Four still images of the sample will be captured from the camera (i.e. 0, 90, -90, and 180 degrees). These images will be used to center the loop. The loop must be on the image for the process to work. In particular, the z position must have the loop in the field of the image as you rotate the sample.

A good protocol would be to auto align on the bottom camera and then the top camera. If the sample has not been centered properly, then perform the final alignment by using the point and click function.

Remember, the system is attempting to optically center the loop, NOT YOUR SAMPLE. So, it will work best if your sample is in the center of the loop.

Click-Alignment

The center of red-cross line is the presumed beam center. After previous **Energy Change and Beam Alignment Procedure**, the x-ray beam should have been centered to this center. Click **left-mouse-button** at crystal center, program will automatically move crystal center to red-cross line center. This click-alignment procedure should be done on different crystal orientation by clicking on +X, -X, +Y, -Y buttons.

Fine-Adjustment

Click **Up-Down-Left-Right** buttons to fine tune the crystal position to get crystal center as close as possible to red-cross line center.

MAD Experiment

- 1) Change the energy to the desired heavy atom absorption edge (K_{α} or L_3) energy, which must be between 6 Kev and 30 Kev and align the beam by following Energy Change and Beam Alignment Procedure.
- 2) Put on sample crystal and align it.
- 3) Make sure the Rontec fluorescence detector is aligned (by default) and power supply is ON.
- 4) Run fluorescence scan:
 - 1). Change window to sergui >MAD tab.
 - 2). From Periodic Table, choose the desired heavy atom and correct edge.
 - 3). Change the transmission to $20\% \sim 50\%$.
 - 4). Click Run Scan and wait until the scan is done (about 1 minute) and the scan plot is displayed. Timing shutter will be automatically closed after scan.
 - 5). Click **Analysis** to run **Benny-Chooch** programs.
 - 6). **Benny-Chooch** window will start automatically to calculate f' and f'. f' and f' plots will be displayed for the scan.
 - 7). Close the plot window and "**Hit return to Close**" the **Benny** window. The calculated inflection wavelength, peak wavelength and their f' and f' will be automatically updated and displayed to the **sergui** window. Type in remote energy about 50 100 ev beyond the edge energy in the window to calculate its f' and f'.
- 5) Change the transmission to desired number for real data collection.
- 6) Setup data collection with calculated inflection, peak and remote energies.
- 7) After data collection and processing, use the program **XPREP** to calculate Patterson map by following **Patterson Map Calculation with Program XPREP**.
- 8) To search multiple heavy atom sites, user may use following programs by **Crystallographic Software**.

```
sgxpro # run either solve or shelxd to search heavy atom.
```

BnP # run PHASES and SnB packages GUI

hkl2map # run shelxc and shelxd

9) To do fluorescence scan background determination manually, type following command:

```
Example: Se K-edge scan plot Se K scan.001
```

\$/usr/local/Chooch/chooch fluorescence.py Se K Se K scan.001

Data Collection

1. Setup file names and archive directory:

From **sergui** > **Collect**,

a. Create **Sample ID** (file name): lysozyme

Template: lysozyme 1 #.####

b. Create Archive Directory:

Template: /u9/staff/08 08 05 SERCAT staff

2. Collect Single:

From sergui.py > Collect > Collect Single

a. Set following target values:

Transmission

Distance

Omega

Scan Width

Exposure Time

b. Click Collect to start collecting single frame:

What happens: i. Move Distance to target position (take time to move).

ii. Move Omega to target position.

iii.Start exposure.

c. Image will be displayed on marccd program and file name will be lysozyme 1 s.####

3. Collect Run of single wavelength data set:

From sergui.py > Collect > Collect Strategy

a. Set following target values:

Exposure Time

Starting Angle (default is 0, click Use Current to change)

Scan Width

Number of Frames/Run

Detector Distance (default is current position, click Use Current to change)

Starting Energy (default is current energy, click Use Current to change)

b. Click Generate Runlist

- c. Go to page > Collect > Collect Runs
- d. Click Collect to start data collection
- e. Click **Abort** to abort data collection

4. Collect Run of MAD data set:

From sergui.py > Collect > Collect Strategy

a. Set following target values:

Exposure Time

Starting Angle (default is 0, click Use Current to change)

Scan Width

Number of Frames/Run

Detector Distance (default is current position, click Use Current to change)

Starting Energy (default is current energy, click Use Current to change to Peak) **Second Energy** (default is disabled, click Use in Data Collection to change to

Inflection)

Third Energy (default is disabled, click Use in Data Collection to change to

Remote)

Inverse Beam (default is No, click Y to enable 180 degree beam inverse)

Wedge Size (Set wedge size for data collection, click Y for Wedge in Angle or

Wedge in Energy)

b. Click Generate Runlist

c. Go to page > Collect > Collect Runs

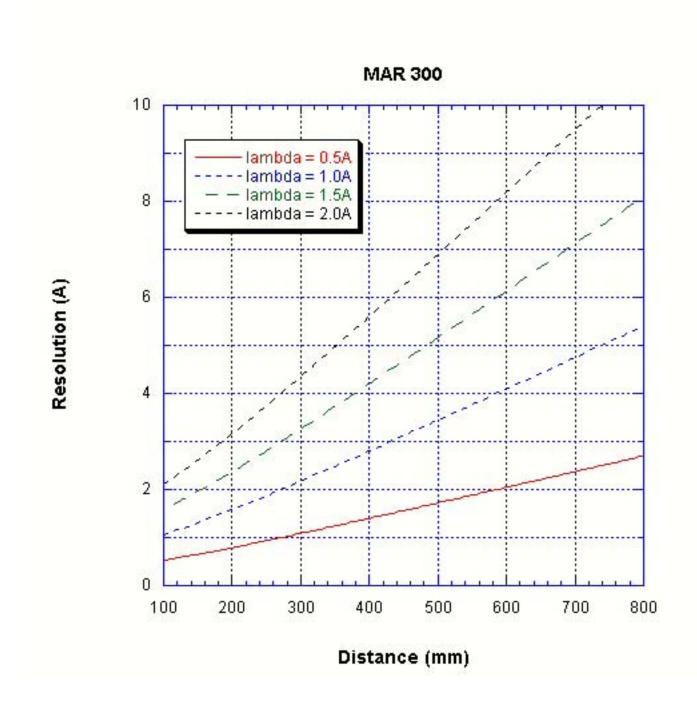
- d. Click Collect to start data collection.
- e. Click **Abort** to abort data collection
- 5. For MAD experiment, make sure the three wavelengths were set correctly for data run.
- 6. If the beam is dumped during normal data collection,
 - 1). Click Abort.
 - 2). After beam current is restored, open the A shutter on PSS manually.
 - 3) Set the correct next frame number and click "Collect" to continue data collection.

Example:

- If beam was dumped at frame #90, user noticed and stopped data collection at frame #94, then next frame number would be #95.
- Frame 90 to frame 94 are empty frames.
- After beam is restored, set the next frame number to 90 to start from the first empty frame. User should not change other Phi, Omega, Kappa values.
- Click Collect to start.

Detector Resolution and Exposure Time

ID beamline and MAR300 detector resolution:



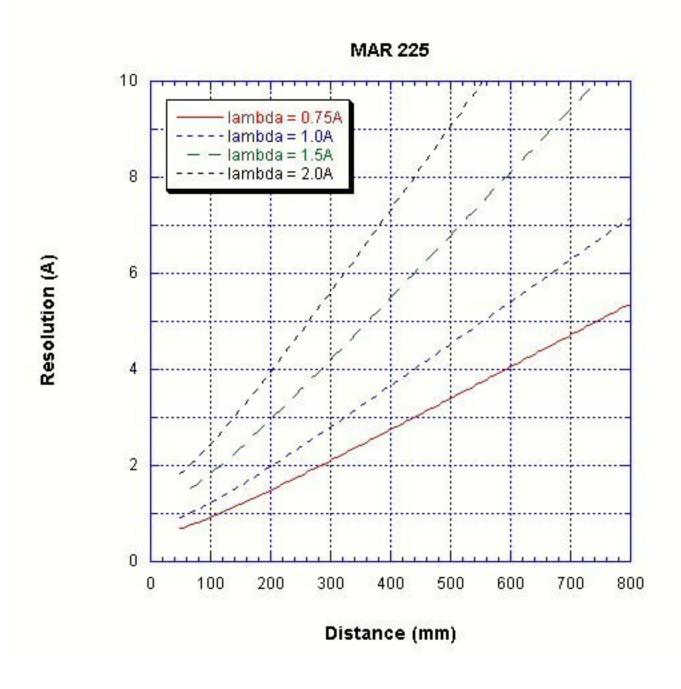
ID beamline and MAR300 detector exposure time

A new version of the MAR remote server software has been installed. The new software reduces the overhead for data collection and increase the number of frames per minute that can be collected.

Exposure Time (sec)	Number of Frames per Minute
1*	11
2*	11
3 *	11
4	9
5	8
6	7
7	6
8	5.5
9	5
10	4.5

- * There is no difference in data collection time for exposures of 1, 2, or 3 seconds. Therefore, for the best data quality, attenuate to allow for 3 second exposures times.
 - The effective overhead time for is approximately 3.1 seconds.

BM beamline and MAR225 detector resolution:



BM beamline and MAR225 detector exposure time

A new version of the MAR remote server software has been installed. The new software reduces the overhead for data collection and increase the number of frames per minute that can be collected.

Exposure Time (sec)	Number of Frames per Minute
1	15.5
2	11.5
3	10
4	8.5
5	7.4
6	6.5
7	6.0
8	5.5
9	5
10	4.5

• The effective overhead time for is approximately 3.1 seconds.

Data Reduction

XGEN, HKL2000, MOSFLM were installed in data processing computers idhkl35, idhkl38, idhkl39, idc23

Run XGEN:

From data processing computer:

bash > cd data-directory # change into data directory

bash> xgenproc [-dns] [-cfhipxy<val>] [dirn]

i.e. d,n,s are Booleans and the other - options all take values # and an option that doesn't begin with a minus is a directory name

- -c*) # specify the number of spots to use
- -d*) # compress images after processing
- -f*) # specify a sample image name
- -h*) # specify the last image number to use
- -i*) # specify an International Tables #
- -n*) # means do NOT recenter after indexing
- -p*) # specify an image prefix
- -s*) # flip the sign of the stepsize
- -x*) # specify the beam Xcenter
- -y*) # specify the beam Ycenter
- *) # specify the data directory

for example:

bash > xgenproc -x2025 -v1998 # reset beam center to (2025, 1998) pixel

- 1. XGEN will create directory **xgena** (or xgenb, xgenz) under data directory.
- 2. XGEN will automatically run through

Peak search Autoindexing Integration Scaling

- 3. Even though XGEN can autoindex single image frame as what HKL2000 does, it will work better with multiple data frames (consecutive or separated).
- 4. XGEN is sensitive to the beam center for successful autoindex. User may change beam center to correct position with parameters.
- 5. XGEN will create log file datafilename.xlg under xgena
- 6. XGEN will create datafilename.sca output data as .sca format
- 7. XGEN will create datafilename.asc output data with anomalous ON as .sca format

Data Reduction

Run HKL2000: From data processing computer: bash > cd data-directory # change into data directory bash> HKL2000 If user wants to use special scalepack for different use, please contact staff scalepack16m scalepack8m scalepackmanyframes scalepackribo scalepackvirus for example: scalepack for Virus project bash> cp /usr/local/bin/scalepack /usr/local/bin/scalepacknorm bash> cp /usr/local/bin/scalepackvirus /usr/local/bin/scalepack **Run Mosflm:** From data processing computer: bash > cd data-directory # change into data directory For ID beamline data from mar300 detector: bash> ipmosflm # start MOSFLM program > detector marccd > limits xmin 0 xmax 150 ymin 0 ymax 150 xscan 150 yscan 150 > template yourdataset.#### > image 1 $> g_0$ For BM beamline data from mar225 detector: bash> ipmosflm # start MOSFLM program > detector marccd reversephi # reverse phi convention for BM > limits xmin 0 xmax 150 ymin 0 ymax 150 xscan 150 yscan 150 > template yourdataset.#### > image 1

 $> g_0$

Heavy Atom Search, Phasing, Maps

Run SGXPRO: (check SER-CAT Software Tutorial)

SGXPRO is an application software suite, developed at University of Georgia, for solving X-ray crystallographic structures of biological macro-molecules.

SGXPEO uses user-friendly GUI for parameter input and is able to run all the procedure as pipeline after user setup.

SGXPRO can be distributed to academic users free.

Heavy atom sites searching:
 Phasing (SAD or MAD):
 SHELXD and/or SOLVE
 ISAS and/or SOLVE

3). Auto-tracing: **RESOLVE**

Start program: bash> sgxpro

Run BnP: (check SER-CAT Software Tutorial)

BnP is the Buffalo and Pittsburgh interface, which is combining **SnB** and **Phases** suite for complete protein phasing.

Start program: bash> BnP

Run CCP4:

Start program: bash> ccp4i

Run HKL2MAP / SHELX: (check SER-CAT Software Tutorial)

Start program: bash> hkl2map

Run CNS:

Start program: bash> cns web

Run O:

Start program: bash> lin ono

Run XtalView:

Start program: bash> xtalmgr

FAO

1. MARCCD program is frozen.

- 1) slogin mar300
- 2) From any shell window, type following command

bash > ps -ax | grep marccd

3) Kill the marced program with shown PID from last command.

bash> kill -9 xxxxx

- 4) Restart **marccd** program from idc23 computer
- 5) Always start **sergui** program after **marccd** program.

2. Remote control box for Phi, Kappa and Z does not work.

- 1) Push down the Phi 0 90 180 270 four buttons at the same time
- 2) From marced program, take a single exposure.
- 3) After exposure, the remote should be activated and ready to use.

3. Could not find data in archive directory set from marced program.

- 1) Check the data collection setup, make sure the archive setup is shown like following format. cp %s /u2/id5/SERCAT/
- 2) Before start the data collection, user must manually create all the directories from shell window. The marced program can not create directory automatically.

4. Could not mount/umount user's firewire drive.

- 1) Check the Firewire Drive Mounting Procedure.
- 2) Check with staff.

5. When starting sergui.py, error message shows: "Socket connection failed,".

- 1) Go to idc10 computer, from any shell window,
 - Type: \$camera server.py
- 2) If step 1) failed, go to video server box (in beamline rack), power reboot.

6. Detector does not move.

- 1) Go to motor> window.
- 2) motor> resync det distance

7. X, Y, Z, Omega do not move.

1) Motor> resync Z